

5 contact between these two types of cells. (2 e 6 , 24-well plates, Costar). Marrow cells were plated in the lower chambers and 5TGM 1 myeloma cells (2e 3) were then plated in either lower (direct contact) or upper (no contact) chambers.

Organ Cultures of ⁴⁵Ca-labelled Fetal Rat Long Bones

10 Conditioned media harvested from 5TGM1 cultures were assayed for bone- — resorbing activity by organ cultures of ⁴⁵Ca-labelled fetal rat long bones as described previously (Mbalaviele 1995). Pregnant rats were injected with 250 uCi of ⁴⁵Ca (New England Nuclear) on the 18th day of gestation. Radius and ulna bone shafts were obtained from 19-day fetuses by microdissection, and precultured for 24 h in BGJ
15 medium (Sigma) supplemented with 0.1 % BSA between air and liquid-phase on stainless mesh grids. Bones were then cultured in the presence of conditioned media (50% v/v) or in control medium for 120 hours. The media were changed once at 48 hours. At the end of the culture, bones were incubated in ice-cold 5% trichloroacetic acid for 2h , and ⁴⁵Ca radioactivity in bones and media determined in a liquid
20 scintillation counter. Bone resorption was quantitated as the percentage of ⁴⁵Ca released into the medium from bones as calculated by: (45Ca count in medium)/ (45Ca count in medium and bone) x 100.

Co-culture of 5TGM1 Myeloma Cells with Mouse Stromal Cell Line ST2 Cells

25 ST2 cells (0.5 e 6) and 5TGM1 (4 e 6) cells were plated together onto 60-mm culture dishes (Beckton Dickinson) in 10% FBS-supplemented IMDM and cultured overnight, washed with serum-free IMDM twice. and incubated in 5 ml of serum-free IMDM. After 48 h, conditioned media were harvested and stored at -70 ° C until use.

30 Effect of mAb PS2 to VLA-4 on serum IgG2b elevation in 5TGM1-bearing mice

Mice were injected with 1 e 5 5TGM1 cells, which were allowed to colonize the bone marrow. Mice were split into two groups of three, one serving as a control group, and the second treated biweekly beginning on day 8 with 80 ug mAb PS/2 (4 mg/kg). Levels